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Cholinergic gating of improvement of tactile acuity induced by peripheral tactile stimulation

Barbara Bliem [a,b,*], Martin Tegenthoff [c], Hubert R. Dinse [a]

[a] *Institute for Neuroinformatics, Theoretical Biology, Neural Plasticity Laboratory, Ruhr-University Bochum, 44801 Bochum, Germany*
[b] *International Graduate School of Neuroscience (IGSN), Ruhr-University Bochum, Germany*
[c] *Department of Neurology, BG-Kliniken Bergmannsheil, Ruhr-University Bochum, Germany*

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Abstract

As shown in many animal experiments, cholinergic mechanisms participate in *N*-methyl-D-aspartate (NMDA) receptor-dependent neuroplasticity. Acetylcholine is thought to play a similar role in humans, where it modulates attention and learning. Here, we tested the cholinergic action on non-associative learning in the tactile domain. We studied the influence of scopolamine, a cholinergic antagonist, on changes in tactile acuity as induced by peripheral tactile coactivation. Coactivation is a non-associative tactile learning protocol and has been shown to improve tactile two-point discrimination of the stimulated finger in addition to selective changes of cortical processing. Under placebo conditions, tactile two-point discrimination was improved on the stimulated index finger. After application of scopolamine, tactile improvement was completely eliminated and tactile acuity was even impaired. No drug effects were found on the left index finger indicating that the drug had no effect on performance per se. The current results provide further evidence that in humans cholinergic mechanisms are also involved in non-associative learning induced by passive stimulation protocols.
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Animal evidence suggests that the expression of cortical sensory plasticity is gated by neuromodulators, particularly acetylcholine plays a crucial role (ACh) [16]. Central cholinergic neurotransmission through muscarinic receptor activation contributes to learning and memory formation and influences long-term potentiation (LTP) [3,4,11,17,20], most likely by enhancing NMDA currents [11].

ACh from the basal forebrain has been demonstrated to serve an important function in attention, arousal, learning and memory, as well as the hypothesized neural substrates of these behavioural phenomena in a wide variety of experimental paradigms [10,17]. For example, in the auditory system, both learned behavioural tone discrimination and the suspected neural correlates of auditory conditioning are similarly modulated by manipulations of the cholinergic system—disruption of normal cholinergic activity impairs behavioural auditory conditioning and neural plasticity, whereas enhancement of cholinergic activity facilitates behavioural learning and plasticity [10,26]. Consequently, the intactness of the basal forebrain cholinergic system is a crucial prerequisite for the expression of cortical map plasticity. Similarly, the disruption of basal forebrain cholinergic function was found to impair motor learning and inhibit cortical reorganization of motor maps [1]. In humans, the impairment of the cholinergic system by application of scopolamine, an ACh antagonist, was shown to suppress experience-dependent plasticity adding support for the role of cholinergic modulation of learning and memory [19,23].

To study cholinergic and thus neuromodulatory influences on cortical plasticity and learning in the tactile domain evoked by passive stimulation, we here applied a peripheral tactile coactivation protocol [21]. This protocol is based on task-unrelated, passive and therefore unattended stimulation inducing an improvement in tactile acuity that is paralleled by an enlargement and shift of cortical representations of stimulated skin sites

* Corresponding author at: Department for Neuroinformatics, Theoretical Biology, Neural Plasticity Laboratory, Ruhr-University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany.
E-mail address: barbara.bliem@rub.de (B. Bliem).

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[6,7,13,14]. We assessed the effects of scopolamine premedication to modulate the typically observed stimulation-induced improvement of tactile discrimination performance.

We tested 13 right-handed subjects of both sexes (5 male, 8 female, mean age 24.9 years, S.D. 4.2) in a randomized double blind, cross-over design. The study was performed in accordance with the Declaration of Helsinki. The subjects gave their written consent, and the protocol was approved by the local ethical committee of Ruhr-University Bochum. General exclusion criteria were a history of neurological and psychiatric disorders, chronic or acute disease and the intake of drugs affecting the central nervous system.

First, discrimination thresholds of the index fingers of both hands were measured using the method of constant stimuli in a simultaneous spatial two-point discrimination task as described previously to assess tactile acuity [6,7,13–15]. In this task seven pairs of needles with separation distances between 0.7 mm and 2.5 mm were used. For controls and to assess false alarm rates, zero distance was tested by using a single needle. Subjects were instructed that there were single needles for control, but not how often they were presented. The needles were placed on a rotable disc allowing to switch rapidly between distances. The disc was installed on a plate that could be moved up and down. The test finger came in contact with the needles whenever the plate was moved down. The subject had to decide immediately after touching the needles whether he or she had the sensation of one or two tips. No feedback was given. Each distance was tested eight times in a randomized order, resulting in 64 trials per session. To obtain stable discrimination thresholds four sessions were done before peripheral tactile stimulation. The summed responses were plotted against distance as a psychometric function for absolute threshold and fitted by a binary logistic regression. Threshold was taken from the fit at the distance where 50% correct responses was reached. To provide a direct test for a bias-free discrimination index, we assessed false alarm and hit rates and calculated the d' prime values [27].

Secondly, transdermal patches containing either scopolamine (Scopoderm TTS®, Novartis) (1.5 mg) or placebo were placed behind the ear of each subject on different sessions separated by at least 2 weeks. Approximately 3 h following placement of each patch, peripheral tactile stimulation was applied. At this time, plasma concentrations reach approximately 50 pg/ml, a threshold value required for appropriate CSF levels and therefore therapeutic effects such as prevention of motion sickness [12].

The protocol of peripheral tactile coactivation was the same as in our previous studies aiming at coactivating a large number of receptive fields on the tip of the index finger in a Hebbian manner to strengthen their mutual interconnectedness [8]. Stimulation was applied to the tip of the right index finger. Tactile stimuli were drawn from a Poisson process at interstimulus-intervals between 100 ms to 3000 ms with a mean frequency of 1 Hz. To apply stimulation, a small device consisting of a small solenoid with a diameter of 8 mm was used, which was taped to the right index finger. Laser vibrometer measurements revealed that the actual amplitude of the probe movement was about 100 μm. Pulses were recorded in MP3 format and played back via a portable player allowing unlimited mobility of the subjects during peripheral tactile stimulation. The duration of stimulation was 3 h, while all subjects resumed their normal day's work. After stimulation another session of two-point discrimination was performed. In addition, the index finger contralateral to stimulation was tested before and after intervention in order to exclude unspecific side effects of the drugs (for experimental setup see also Fig. 1).

A brief standard questionnaire assessing drowsiness, presence of jitters, dryness of the mouth and/or eye, dizziness, restlessness, and confusion was given to participants at the end of the experiment to evaluate drug-related side. Subject had to report verbally whether after application of scopolamine they experienced any changes in the given parameters.

For statistical analysis, a repeated measures ANOVA (rmANOVA) with the factors DRUG (scopolamine, placebo) × SESSION (s1–s4, post) was performed.

Subjects tolerated the administration of scopolamine well. Only one out of 13 participants experienced slight drowsiness at the end of the experiment after application of scopolamine.

In this study we observed a drug-dependent change in discrimination thresholds as induced by peripheral tactile stimulation. The rmANOVA with the factors DRUG × SESSION revealed a significant interaction ($F(4,48) = 7.495$, $P < 0.001$). In placebo-controlled subjects, discrimination thresholds prior to peripheral tactile stimulation were stable ($P > 0.05$). Discrimination thresholds after peripheral tactile stimulation were significantly lower compared to thresholds in the baseline

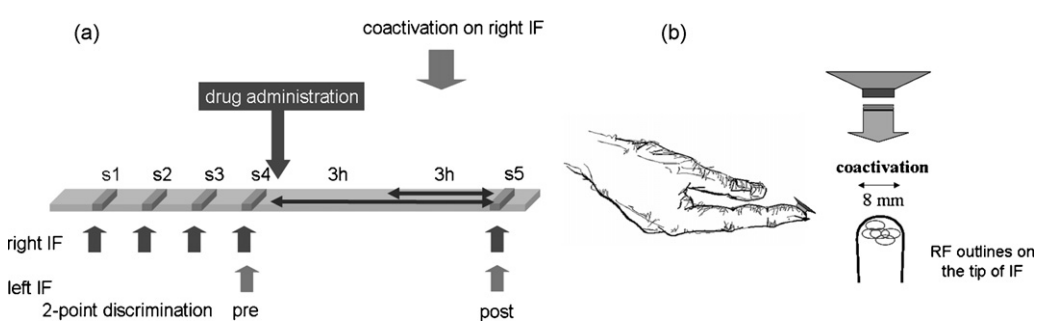

Fig. 1. Experimental design: session 1–4 (s1–s4) served to create a stable discrimination performance for the right index finger (IF). The left IF was tested as control at s4 (pre-stimulation) and after stimulation (session s5, post). Transdermal patches containing either scopolamine or placebo were applied approximately 3 h prior to coactivation. (B) Application of tactile coactivation. A small solenoid with a diameter of 8 mm was mounted on the tip of the right IF to coactivate the receptive fields (RFs) representing the skin portion under the solenoid (50 mm^2).

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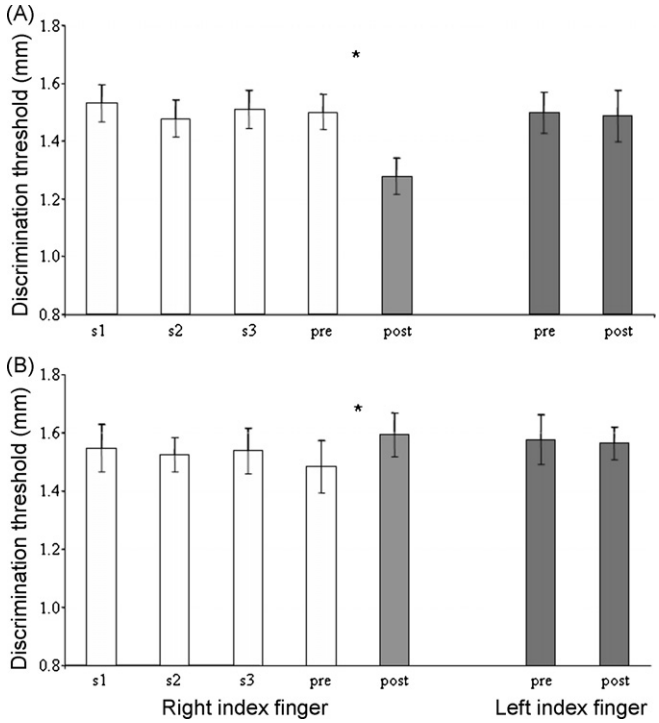

Fig. 2. Psychophysical effects of tactile coactivation. (A) Placebo group. Discrimination thresholds were stable before intervention (s1–s4). After coactivation, discrimination thresholds on the right IF were significantly reduced (indicated by the asterisk, $P < 0.05$), whereas thresholds of the left IF remained unaffected. (B) Scopolamine. Application of scopolamine completely eliminated the coactivation-induced improvement in two-point discrimination. Discrimination thresholds were significantly increased comparing pre and post measurement (indicated by the asterisk, $P < 0.05$). No changes were observed for the non-stimulated left IF. Bars represent standard error.

sessions ($P < 0.05$). After peripheral tactile stimulation, psychometric functions showed a significant shift of thresholds toward smaller separation distances. Discrimination thresholds decreased from 1.52 mm in the initial measurement (pre) to 1.29 mm after stimulation (paired samples *t*-test $P < 0.001$) (Fig. 2). As a control, and to demonstrate the specificity of stimulation-induced changes, we measured thresholds of the left IF, which was not stimulated. Here thresholds remained unchanged (rmANOVA with the factor SESSION pre–post, $F(1,12) = 0.000$, $P = 0.994$).

In contrast, application of scopolamine not only eliminated stimulation-induced improvement in tactile acuity but lead to an impairment of discrimination performance after coactivation. Absolute thresholds significantly increased from 1.47 mm to 1.60 mm (paired samples *t*-test pre–post $P = 0.018$). However, tactile discrimination thresholds after peripheral tactile stimulation only differed between the post and the immediate pre measurement (s5). With respect to discrimination thresholds, we observed no difference between the first three baseline sessions and the session after peripheral tactile stimulation ($P > 0.05$).

Given that this impairment was not due to non-specific side effects exerted by scopolamine it was important to show that the drug did not affect spatial discrimination per se, which was indicated by the lack of changes of thresholds on the left, non-stimulated IF ($F(1,12) = 0.002$, $P = 0.968$).

Further, false alarm rates were zero before intervention and remained stable after stimulation for both experimental conditions. We observed a significant DRUG × SESSION interaction for the d' prime value ($F(1,11) = 10.395$, $P = 0.008$). A paired samples t test revealed a significant increase in d' prime value for the placebo group ($P = 0.012$). After application of scopolamine, there was a significant reduction in d' prime value after stimulation ($P = 0.038$).

Our results indicate that in humans cholinergic mechanisms play an essential role in cortical plasticity evoked by passive stimulation protocols. We found that application of a cholinergic antagonist strongly affected the stimulation-induced improvement in tactile acuity resulting in a blockage of non-associative tactile learning or even impairment in spatial tactile discrimination.

An important confound when investigating drug effects in humans is sedation, which has been reported frequently after drugs such as scopolamine. As shown consistently many times before, stimulation-induced improvement in discrimination performance is highly specific to the stimulated finger with no transfer to the fingers of the other hand [6,7,13–15]. We therefore used the performance of the index finger of the left hand to measure the specificity of the observed drug effects. Scopolamine had no effect on spatial discrimination performance per se, which together with the consistency of the effects across subjects supports the specific nature of the drug influence observed for the right index finger. Since scopolamine patch placement results in maximal plasma levels at about 24 h [12] and in our study the patch was kept for a maximum of 7 h, the relative paucity of side effects is not unexpected.

As previously shown, improvements in tactile acuity induced by peripheral stimulation are blocked by NMDA receptor antagonists [2,13,14]. It has therefore been hypothesized that NMDA receptor activation plays an important role which points towards a possible involvement of long-term potentiation (LTP) and long-term depression (LTD). As shown consistently, cholinergic action successfully modulates LTP [3,4,11,17,20], most likely by enhancing NMDA currents [11]. The current results suggesting a block of LTP-like changes in tactile acuity by application of a cholinergic antagonist in humans are therefore in line with previous in vitro and in vivo experiments.

Further, Dinse and coworkers demonstrated that changes in tactile acuity as induced by peripheral tactile stimulation are paralleled by cortical map reorganization. In detail, improvement in tactile acuity was positively correlated with an enlargement of cortical representations of the stimulated finger [2,13,14] suggesting a causal relation. On the other hand, lesions in the basal forebrain cholinergic system are known to block cortical plasticity including the robust topographic reorganizations that follow motor skill learning, peripheral denervation and digit amputation [1,9,25]. Juliano et al. observed that total basal forebrain lesions not only blocked the expected expansion of remaining intact digits following digit amputation in the cat forelimb, but also even led to a shrinkage of the sensory representations. Their results indicate that the basal forebrain cholinergic system might be instrumental to maintain normal representations within the cortex [9].

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The observation that perceptual improvement could be obtained using a passively applied unattended stimulation protocol is in line with recent studies in the visual system. For example, perceptual learning was induced by repetitive exposure to stimuli that were below the threshold of visibility and that were irrelevant to the central task [24]. These findings as well as ours described here show that perceptual learning can take place not only under training conditions, but also in situations of unattended and passive sensory stimulation. However, the stimulation protocol was suprathreshold, raising the possibility that subjects were unable to avoid attending to the stimulation. Further, stimulation that effectively controls the timing of applied stimuli, as it is the case in peripheral tactile stimulation, might short cut the role of attention by producing the same conditions as attention, such as synchronous firing or increased probability of firing in specific temporal order among groups of neurons [5,22].

For learning to occur, stimulus-related responses that are under normal conditions insufficient to drive changes must surpass a so-called learning threshold. Attention and reinforcement have been proposed to enhance stimulus driven responses. In addition, the optimization of sensory inputs by synchronization or multisensory stimulation, or magnetic/electrical stimulation, can serve to enhance input signals and by doing so support learning [21]. These mechanisms are most likely mediated by neuromodulatory processes. For example, cholinergic inputs are thought to be the source of the top-down signals that serve to sharpen or enhance specific bottom-up inputs [18].

The key role of cholinergic activation in mediating perceptual improvement was confirmed in the current study. First, the action of the cholinergic antagonist scopolamine on mechanisms of synaptic plasticity and cortical representations provides one possible explanation for the blockage of non-associative tactile learning. In line with the reports of Juliano et al. [9] we speculate that scopolamine not only blocked the typically observed enlargement of the cortical representation of the stimulated finger, but even impaired tactile acuity by decreasing the cortical map size. Secondly, the cholinergic system is thought to provide the neural substrate in cognitive functions such as arousal and attention. By interfering with attentive mechanisms, scopolamine might also have blocked or even reversed the impact of peripheral tactile stimulation.

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